## PENDING CLAIMS 48-100 & 102-182 STAVRIANOPOULOS ET AL., U.S. PATENT APPLICATION SERIAL NO. 08/486,070 FILED JUNE 7, 1995

48. A composition of matter comprising:

a transparent non-porous or translucent non-porous system containing a fluid or solution, which system comprises:

- (i) a solid support; and
- (ii) a double-stranded oligonucleotide or polynucleotide which is directly or indirectly fixed or immobilized to said solid support wherein one of the strands produces a soluble signal generated or generatable from a chemical label or labels which comprise a signalling moiety or moieties.
- 49. The composition according to claim 48, wherein said solid support is contained within the transparent non-porous or translucent non-porous system.
- 50. The composition according to claim 48, wherein said solid support is porous or non-porous.
- 51. The composition according to claim 50, wherein said porous solid support comprises a porous polymeric material.
- 52. The composition according to claim 51, wherein said porous polymeric material is selected from the group consisting of dextran, cellulose and nitrocellulose.
- 53. The composition according to claim 50, wherein said non-porous solid support is selected from the group consisting of siliceous matter and non-porous polymeric material.
- 54. The composition according to claim 53, wehrein said siliceous matter comprises glass or a glass-coated surface.
- 55. The composition according to claim 133, wherein said plastic or plastic-coated surface is selected from the group consisting of polyethylene, polypropylene, polystyrene and epoxy.

Serial No. 08/486,070

Filed: June 7, 1995

Page 2 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 56. The composition according to claim 48, wherein said system is selected from the group consisting of a well, a tube, a cuvette and an apparatus that comprises a plurality of said wells, tubes or cuvettes.
- 57. The composition according to claim 56, wherein said well comprises a microtiter well.
- 58. The composition according to claim 56, wherein said wells in the apparatus comprise microtiter wells.
- 59. The composition according to claim 48, wherein said solid support and said system are composed of the same materials.
- 60. The composition according to claim 48, wherein said solid support and said system are composed of different materials.
- 61. The composition according to claim 48, wherein one of said oligonucleotide or polynucleotide strands is directly or indirectly fixed or immobilized to the solid support.
- 62. The composition according to claim 61, wherein said oligonucleotide or polynucleotide strand is directly fixed or immobilized to the solid support by sandwich hybridization.
- 63. The composition according to claim 48, wherein said double-stranded oligonucleotide or polynucleotide is selected from the group consisting of DNA, RNA and a DNA-RNA hybrid, or a combination of any of the foregoing.
- 64. The composition according to claim 48, wherein said label or labels are the signalling moiety or moieties.
- 65. The composition according to claims 48 or 64, wherein said label or labels are directly attached to the oligonucleotide or polynucleotide.

Serial No. 08/486,070

Filed: June 7, 1995

Page 3 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- The composition according to claims 48 or 64, wherein said label or labels 66. are indirectly attached to the oligonucleotide or polynucleotide.
- The composition according to claim 66, wherein said label or labels are 67. indirectly attached to the oligonucleotide or polynucleotide through the formation of a complex.
- The composition according to claim 67, wherein said complex is selected 68. from the group consisting of biotin and avidin, biotin and streptavidin, a sugar and lectin, and an antigen and an antibody.
- The composition according to claim 66, wherein said label or labels are 69. indirectly attached to the oligonucleotide or polynucleotide through a bridging moiety.
- The composition according to claim 48, wherein the signalling moiety or 70. moieties of said label or labels are directly or indirectly attached thereto.
- The composition according to claim 48, wherein said signalling moiety or moieties are selected from the group consisting of an enzyme, a co-enzyme, a chelating agent, a chromagen, a fluorescent agent and a chemiluminescent agent.
- The composition according to claim 48, wherein said soluble signal is 72. generatable from a chromagen, or by fluorescence or chemiluminescence.
- The composition according to claim 72, wherein said soluble signal is 73. indirectly generatable by an enzyme or enzymatic reaction.
- The composition according to claim 48, wherein said soluble signal is 74. detectable by a technique selected from the group consisting of photometric techniques and colorimetric techniques.
- The composition according to claim 74, wherein said photometric techniques 75. comprise spectrophotometric techniques.

Serial No. 08/486,070

Filed: June 7, 1995

Page 4 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 76. The composition according to claim 48, wherein said soluble signal is selected from the group consisting of a colored product, a chemiluminescent product and a fluorescent product.
- 77. (Amended) A composition of matter comprising:

a transparent non-porous or translucent non-porous system containing a fluid or solution, which system comprises:

a double-stranded oligonucleotide or polynucleotide which is directly or indirectly fixed or immobilized to said system wherein one of the strands produces a soluble signal generated or generatable from a chemical label or labels which comprise a signalling moiety or moieties.

- 78. The composition according to claim 77, wherein said non-porous system is selected from the group consisting of siliceous matter and non-porous polymeric material.
- 79. The composition according to claim 78, wherein said siliceous matter comprises glass or a glass-coated surface.
- 80. The composition according to claim 134, wherein said plastic or plastic-coated surface is selected from the group consisting of polyethylene, polypropylene, polystyrene and epoxy.
- 81. The composition according to claim 77, wherein said system is selected from the group consisting of a well, a tube, a cuvette and an apparatus that comprises a plurality of said wells, tubes or cuvettes.
- 82. The composition according to claim 81, wherein said well comprises a microtiter well.
- 83. The composition according to claim 81, wherein said wells in the apparatus comprise microtiter wells.

Serial No. 08/486,070

Filed: June 7, 1995

Page 5 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 84. The composition according to claim 77, wherein one of said oligonucleotide or polynucleotide strands is directly or indirectly fixed or immobilized ot said system.
- 85. The composition according to claim 84, wherein said oligonucleotide or polynucleotide strand is directly fixed or immobilized to the system by sandwich hybridization.
- 86. The composition according to claim 48, wherein said double-stranded oligonucleotide or polynucleotide is selected from the group consisting of DNA, RNA and a DNA-RNA hybrid, or a combination of any of the foregoing.
- 87. The composition according to claim 77, wherein said label or lables are the signalling moiety or moieties.
- 88. The composition according to claims 77 or 87, wherein said label or labels are directly attached to the oligonucleotide or polynucleotide.
- 89. The composition according to claims 77 or 87, wherein said label or labels are indirectly attached to the oligonucleotide or polynucleotide.
- 90. The composition according to claim 89, wherein said label or labels are indirectly attached to the oligonucleotide or polynucleotide through the formation of a complex.
- 91. The composition according to claim 90, wherein said complex is selected from the group consisting of biotin and avidin, biotin and streptavidin, a sugar and a lectin, and an antigen and an antibody.
- 92. The composition according to claim 89, wherein said label or labels are indirectly attached to the oligonucleotide or polynucleotide through a bridging moiety.
- 93. The composition according to claim 77, wherein the signalling moiety or moieties of said label or labels are directly or indirectly attached thereto.

Serial No. 08/486,070 Filed: June 7, 1995

Page 6 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

94. The composition according to claim 77, wherein said signalling moiety or moieties are selected from the group consisting of an enzyme, a co-enzyme, a chelating agent, a chromagen, a fluorescent agent and a chemiluminescent agent.

- 95. The composition according to claim 77, wherein said soluble signal is generatable from a chromagen, or by fluorescence or chemiluminescence.
- 96. The composition according to claim 95, wherein said soluble signal is indirectly generatable by an enzyme or enzymatic reaction.
- 97. The composition according to claim 77, wherein said soluble signal is detectable by a technique selected from the group consisting of photometric techniques and colormetric techniques.
- 98. The composition according to claim 97, wherein said photometric techniques comprise spectrophotometric techniques.
- 99. The composition according to claim 77, wherein said soluble signal is selected from the group consisting of a colored product, a chemiluminescent and a fluorescent product.

## 100. An apparatus comprising:

- one or more solution containing means, each comprising a transparent non-porous or translucent non-porous device;
- 2) means for forming a fixed or immobilized double-stranded oligonucleotide or polynucleotide hybrid to a solid support in said device, said hybrid comprising a chemical label or labels attached to one strand of said hybrid, said label or labels comprising a signalling moiety or moieties which are capable of generating a soluble signal; and
- 3) means for producing a quantifiable or measurable soluble signal generatable or generated from said chemical label or labels which comprise said signalling moiety or moieties.

Serial No. 08/486,070

Filed: June 7, 1995

Page 7 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 102. A transparent non-porous or translucent non-porous system containing a fluid or solution, which system comprises:
- (ii) an oligonucleotide or polynucleotide hybridized or hybridizable to an oligo-polynucleotide sequence, said oligonucleotide or polynucleotide in double-stranded form producing a soluble signal generated or generatable from a chemical label or labels which comprise a signalling moiety or moieties; and
- (ii) a solid support having directly or indirectly fixed or immobilized thereto said oligo- or polynucleotide sequence or said oligonucleotide or polynucleotide (i).
- 103. The system according to claim 102, wherein said solid support is contained within said transparent non-porous or translucent non-porous system.
- 104. The system according to claim 102, wherein the solid support is porous or non-porous.
- 105. The system according to claim 104, wherein said porous solid support comprises a porous polymeric material.
- 106. The system according to claim 105, wherein said porous polymeric material is selected from the group consisting of dextran, cellulose and nitrocellulose.
- 107. The system according to claim 104, wherein said non-porous solid support is selected from the group consisting of siliceous material and non-porous polymeric material.
- 108. The system according to claim 104, wherein said siliceous material comprises glass or a glass-coated surface.
- 109. The composition according to claim 135, wherein said plastic or plastic-coated surface is selected from the group consisting of polyethylene, polypropylene, polystyrene and epoxy.

Serial No. 08/486,070 Filed: June 7, 1995

Page 8 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 110. The system according to claim 102, wherein said system is selected from the group consisting of a well, a tube, a cuvette and an apparatus that comprises a plurality of wells or microtitre wells, tubes or cuvettes.
- 111. The system according to claim 110, wherein said well comprises a microtiter well.
- 112. The system according to claim 110, wherein said wells in the apparatus comprise microtiter wells.
- 113. The system according to claim 102, wherein said solid support and said system are composed of the same materials.
- 114. The system according to claim 102, wherein aid solid support and said system are composed of different materials.
- 115. The system according to claim 102, wherein said solid support is capable of indirectly fixing or immobilizing the oligo- or polynucleotide sequence or said oligonucleotide or polynucleotide (i).
- 116. The system according to claim 115, wherein said oligo-or polynucleotide sequence or said oligonucleotide or polynucleotide is indirectly fixed or immobilized to the solid support through the hybridization of a complementary oligo- or polynucleotide sequence.
- 117. The system according to claim 102, wherein said solid support is capable of indirectly fixing or immobilizing the oligonucleotide or polynucleotide.
- 118. The composition according to claim 102, wherein said double-stranded oligonucleotide or polynucleotide (i) or said oligo- or polynucleotide is selected from the group consisting of DNA, RNA and a DNA-RNA hybrid, or a combination of any of the foregoing.
- 119. The system according to claim 102, wherein said label or labels are the signaling moiety.

Serial No. 08/486,070

Filed: June 7, 1995

Page 9 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 120. The system according to claims 102 or 119, wherein said label or labels are directly attached to the oligonucleotide or polynucleotide.
- 121. The system according to claims 102 or 119, wherein said label or labels are indirectly attached to the oligonucleotide or polynucleotide.
- 122. The system according to claim 121, wherein said label or labels are indirectly attached to the oligonucleotide or polynucleotide through the formation of a complex.
- 123. The system according to claim 122, wherein said complex is selected from the group consisting of biotin and avidin, biotin and streptavidin, a sugar and a lectin, and an antigen and an antibody.
- 124. The system according to claim 121, wherein said label or labels are indirectly attached to the oligonucleotide or polynucleotide through a bridging moiety.
- 125. The system according to claim 102, wherein the signalling moiety or moieties of said label or labels are directly or indirectly attached thereto.
- 126. The system according to claim 102, wherein said signalling moiety or moieties are selected from the group consisting of an enzyme, a co-enzyme, a chelating agent, a chromagen, a fluorescent agent and a chemiluminescent agent.
- 127. The system according to calim 102, wherein said soluble signal is generatable from a chromagen, or by fluorescence or chemiluminescence.
- 128. The system according to claim 102, wherein said soluble signal is indirectly generatable by an enzyme or enzymatic reaction.
- 129. The system according to claim 102, wherein said soluble signal is detectable by a technique selected from the group consisting of photometric techniques and colorimetric techniques.

Serial No. 08/486,070

Filed: June 7, 1995

Page 10 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 130. The system according to claim 129, wherein said photometric techniques comprise spectrophotometric techniques.
- 131. The system according to claim 102, wherein said soluble signal is selected from the group consisting of a colored product, a chemiluminescent product and a fluorescent product.
- 132. An apparatus comprising:
  - 1) means for retaining or containing a fluid or solution;
- 2) one or more transparent non-porous or translucent non-porous devices, each comprising a solid support;
- 3) means for forming a fixed or immobilized oligonucleotide or polynucleotide hybrid to said solid support, said hybrid comprising a chemical label or labels attached to said hybrid, said label or labels further comprising a signalling moiety or moieties capable of generating a soluble signal;
- 4) means for quantifying or detecting a soluble signal generatable or generated from said chemical label or labels comprising said signalling moiety or moieties; and
  - 5) fluid or solution.
- 133. The composition of claims 54 or 79, wherein said glass or glass-coated surface comprises porous glass.
- 134. The system of claim 108, wherein said glass or glass-coated surface comprises porous glass.
- 135. The composition of claim 53, wherein said non-porous polymeric material comprises plastic or a plastic-coated surface.
- 136. The composition of claim 78, wherein said non-porous polymeric material comprises plastic or a plastic-coated surface.
- 137. The composition of claim 107, wherein said non-porous polymeric material comprises plastic or a plastic-coated surface.

Serial No. 08/486,070

Filed: June 7, 1995

Page 11 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 138. The composition of claim 48, wherein said system is selected from the group consisting of a well, a tube, a cuvette and an apparatus that comprises a plurality of said wells, tubes or cuvettes, and said solid support is selected from the group consisting of dextran, cellulose, nitrocellulose, glass or a glass-coated surface and plastic or a plastic-coated surface.
- 139. The system of claim 102, wherein said system is selected from the group consisting of a well, a tube, a cuvette and an apparatus that comprises a plurality of said wells, tubes or cuvettes, and said solid support is selected from the group consisting of dextran, cellulose, nitrocellulose, glass or a glass-coated surface and plastic or a plastic-coated surface.
- 140. The composition of claim 48, wherein said system functions as the solid support.
- 141. The composition of claim 77, wherein said system functions as a solid support.
- 142. The system of claim 102, wherein said system functions as the solid support.
- 143. An array of substrate surfaces, said array comprising a plurality of nucleic acid strands fixed or immobilized to said substrate surfaces.
- 144. The array of claim 143, wherein each said substrate surfaces has been treated to enhance fixation or immobilization to the surface.
- 145. The array of claim 144, wherein said treatment is carried out using an amine or amide compound.
- 146. The array of claim 145, wherein said amine compound is selected from the group consisting of duodecadiamine (DDA), polylysine (PPL), aminopropyltriethoxysilane or a combination of any of the foregoing.
- 147. The array of claim 145, wherein said amide compound comprises formamide.

Serial No. 08/486,070 Filed: June 7, 1995

Page 12 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 148. The array of claim 144, wherein said treatment is carried out using a dispersive compound.
- 149. The array of claim 148, wherein said dispersive compound comprises ammonium acetate.
- 150. The array of claim 144, wherein said treatment is carried out using an epoxy compound.
- 151. The array of claim 144, wherein said treatment is carried out using an amine compound and an epoxy compound.
- 152. The array of any of claims 143, 144, 145, 146, 147, 148, 149, 150 or 151, wherein said substrate surface is porous or non-porous.
- 153. The array of claim 152, wherein said porous substrate surface comprises a porous polymeric material.
- 154. The array of claim 153, wherein said polymeric material is selected from the group consisting of dextran, cellulose and nitrocellulose.
- 155. The array of claim 152, wherein said non-porous substrate surface is selected from the group consisting of siliceous matter and non-porous polymeric material.
- 156. The array of claim 155, wherein said siliceous matter comprises glass or a glass-coated surface.
- 157. The array of claim 156, wherein said glass or glass-coated surface comprises porous glass.
- 158. The array of claim 156, wherein said glass or glass-coated surface is selected from the group consisting of wells, depressions, tubes, cuvettes and an apparatus that comprises a plurality of said wells, tubes or cuvettes.

· Serial No. 08/486,070

Filed: June 7, 1995

Page 13 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 159. The array of claim 158, wherein said wells comprise microtiter wells.
- 160. The array of claim 155, wherein said non-porous polymeric material comprises a plastic.
- 161. The array of claim 160, wherein said plastic is selected from the group consisting of polyethylene, polypropylene, polystyrene and epoxy.
- 162. The array of claim 143, wherein said nucleic acid strands are fixed or immobilized directly or indirectly to said substrate surface.
- 163. The array of claim 143, wherein said nucleic acid strands are single-stranded or double-stranded.
- 164. The array of claim 163, wherein said nucleic acid strands are selected from the group consisting of DNA and RNA, a DNA-RNA hybrid, or combinations thereof.
- 165. The array of claim 164, wherein said nucleic acid strands comprise nucleic acid probe sequences complementary to a target nucleic acid sequence of interest.
- 166. The array of claim 143, wherein said nucleic acid probe sequences are unlabeled.
- 167. The array of claim 163, wherein at least one of said double-stranded nucleic acid strands produces a soluble signal generated or generatable from a chemical label or labels comprising a signaling moiety or moieties.
- 168. The array of claim 167, wherein said label or labels are the signaling moiety or moieties.
- 169. The array of claim 168, wherein the signaling moiety or moieties of said label or labels are directly or indirectly attached thereto.

Serial No. 08/486,070

Filed: June 7, 1995

Page 14 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 170. The array of claim 167, wherein said labeled nucleic acid strand comprises a nucleic acid sequence sought to be identified or sequenced.
- 171. The array of claim 163, wherein said label or labels are attached directly or indirectly to one or more nucleotides in said nucleic acid strand.
- 172. The array of claim 163, wherein said label or labels are indirectly attached to one or more nucleotides through the formation of a complex.
- 173. The array of claim 172, wherein said complex is selected from the group consisting of biotin and avidin, biotin and streptavidin, a sugar and a lectin, and an antigen and an antibody.
- 174. The array of claim 167, wherein said label or labels are indirectly attached to one or more nucleotides through a bridging moiety.
- 175. The array of claim 167, wherein said signaling moiety or moieties are selected from the group consisting of an enzyme, a co-enzyme, a chelating agent, a chromagen, a fluorescent agent and a chemiluminescent agent.
- 176. The array of claim 167, wherein said soluble signal is generated or generatable from a chromagen, or by fluorescence or chemiluminescence.
- 177. The array of claim 167, wherein said soluble signal is quantifiable or detectable by a technique selected from the group consisting of photometric techniques and colorimetric techniques.
- 178. The array of claim 177, wherein said photometric techniques comprise spectrophotometric techniques.
- 179. The array of claim 167, wherein said soluble signal is selected from the group consisting of a colored product, a chemiluminescent product and a fluorescent product.

Serial No. 08/486,070

Filed: June 7, 1995

Page 15 (Exhibit B to Declaration of Dr. Dean L. Engelhardt in Support of Possession of Claimed Subject Matter and Novelty of Invention)

- 180. An apparatus which comprises the array of any of claims 143 to 151, or 153 to 179, wherein said substrate surface is porous or non-porous.
- 181. A transparent non-porous or translucent non-porous system capable of retaining or containing a fluid or solution, which system comprises the array of any of claims 143 to 151, or 153 to 179, wherein said substrate surface is porous or non-porous.
- 182. The system of claim 181, wherein said substrate surface is contained within the transparent non-porous or translucent non-porous system.

\* \* \* \* \* \*